Surprising Findings Following a Belgian Food Contamination with Polychlorobiphenyls and Dioxins

Paul J.C. Schepens,[1] Adrian Covaci,[1] Philippe G. Jorens,[1] Luc Hens,[2] Simon Scharpé,[1] and Nik van Larebeke[3]

[1]Toxicological Center and Departments of Medicine and Medical Biochemistry, University of Antwerp, Wilrijk, Belgium; [2]Department of Human Ecology, Free University of Brussels, Brussels, Belgium; [3]Department of Radiotherapy, Nuclear Medicine and Experimental Oncology, University of Ghent, Ghent, Belgium

We found that 12.1% of Belgian export meat samples from chicken or pork, unrelated to the PCB/dioxin crisis from 1999, contained more than 50 ng polychlorinated biphenyls (PCBs)/g fat and that 6.5% of samples contain more than 20 ng/g fat for the sum of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites. Part of this background contamination stems from imported animal feed ingredients (fish flour and grains), sometimes contaminated by recent use of DDT, as can be deduced from the ratio between DDT and its main metabolite, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE). However, after comparing PCB concentrations in fish flour and grains with those found in meat, we suggest that the high concentrations stem from recycled fat. This is the first paper describing background concentrations of PCBs in animal meat from Belgium. *Key words*: animal fat, contamination, DDT, food, polychlorinated biphenyls (PCBs). *Environ Health Perspect* 109:101–103 (2001). [Online 10 January 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p101-103schepens/abstract.html

The Belgian PCB/Dioxin Crisis

In 1999, about 50 kg polychlorobiphenyls (PCBs) and 1 g dioxins were introduced into the animal food chain through approximately 1,500 tons of animal feed containing 60 tons of contaminated fat from a Belgian fat-melting company. This incident caused widespread concern both in and outside Belgium and obliged the Belgian government to take drastic measures to protect public health, including a large-scale food-monitoring program with measurements of PCBs and dioxins in, respectively, over 20,000 and 450 samples from animal feed, animal fat, and various fat-containing food items (*1*). All samples were analyzed in officially accredited laboratories.

Analysis of contaminated foodstuff showed a pattern of PCBs closely matched with a mixture of Aroclor 1254 and 1260 and a consistent pattern of dioxin-like compounds, dominated by polychlorodibenzofurans. These patterns were virtually identical to that in the 1969 Yusho rice poisoning, caused by heat-degraded PCBs (*2*).

Sampling and Methods

Because most of the suspected animal food suppliers and their clients could be traced, all implicated livestock arriving in slaughterhouses were carefully checked and sampled by officially approved veterinarians. All samples were registered, sealed, and labeled. Nonsuspected farms (those that did not obtain animal feed from producers who might have incorporated ingredients from the incriminated fat-melting company) needed for export official governmental certificates. Animals from these farms were checked and sampled in the same manner.

From September to November 1999, the Toxicological Center received and analyzed 1,850 samples of Belgian meat for export (chicken and pork) containing PCBs. Feed ingredients (e.g., grains and fish flour) were provided by the Belgian Federation of Animal Food Processing, the Belgian Ministry of Agriculture, and the only company importing fish flour in Belgium (Comanima NV, Antwerp, Belgium). All feed samples were imported from other countries.

The analysis included three main steps: isolation of lipids and PCBs from the raw material (a matrix-dependent procedure), clean-up on acidified silica gel (44% concentrated sulfuric acid), and determination by gas chromatography with electron capture detection (ECD; HT-8 capillary column) or mass spectrometric detection (DB-5 capillary column). The last two steps were similar for all matrices.

After homogenization, the fish flour, grains, and animal feed (2–5 g) were spiked with 5 ng internal standard (PCB 143) and Soxhlet-extracted with hexane for 4 hr. The extracts were concentrated to 2 mL and cleaned on acidified silica gel.

A representative sample of animal (chicken or pork) fat was cut into small pieces and melted at 80°C for 10 min. An aliquot of 0.5 g fat was weighed, solubilized in hexane, and spiked with 5 ng internal standard (PCB 143). After ultrasonic equilibration for 10 min, the mixture was cleaned on acidified silica gel.

A typical ECD chromatogram (Figure 1) of pork fat contaminated with PCBs and DDTs shows unambiguous identification of all investigated compounds. Mean recoveries for studied compounds were 80% and detection limits ranged between 0.5 and 1 ng/g fat. We assessed methodological accuracy through rigorous internal quality control, which included a daily check of calibration curves and regular analysis with each batch of samples of procedural blanks, spiked blank fat at a concentration of 100 ng/g fat for each PCB congener, and of certified material CRM 350 (PCBs in mackerel oil). After participating in interlaboratory testing organized by the Belgian Ministry of Health (*3*), which included all 22 laboratories involved in PCB analyses during the PCB/dioxin crisis, we concluded that the correct validation procedure was used and the method can be applied to different types of samples.

Background Contamination with Organochlorines

Results, summarized in Table 1, are expressed as the sum of the seven marker PCBs (International Union of Pure and Applied Chemistry numbers 28, 52, 101, 118, 138, 153, and 180). For chicken and for pork, 1.8% of the samples tested contained more than 200 ng PCBs/g fat, the tolerated level imposed on Belgium by the European Union (*4*). Concentrations between 50 and 200 ng PCBs/g fat, considered moderately elevated, were found in 9.1% of chicken and 10.6% of pork samples tested. Meat from older pork (mean age of 30 months) contained significantly ($p < 0.05$) higher concentrations of PCBs (44 ± 38 ng/g fat, $n = 578$) than did young pork (6–7 months of age; 31 ± 72 ng/g fat, $n = 888$). The high variability in the results is due to the fact that samples came from different breeders and food suppliers all over Belgium. Moreover, the percentage of samples with concentrations above 50 ng PCBs/g fat was higher (13.2%) for old pork than for young pork (9.2 %) (Figure 2). These findings indicate that animals accumulate PCBs with age, due to continuous exposure (*5*).

Address correspondence to A. Covaci, Toxicological Center and Departments of Medicine and Medical Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium. Telephone: +32 3 820 2704. Fax: +32 3 820 2722. E-mail: covaci@uia.ua.ac.be

Received 3 July 2000; accepted 5 September 2000.

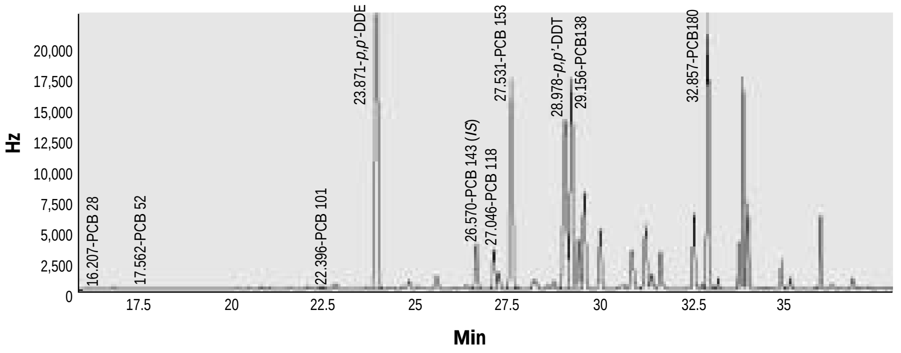

Figure 1. Typical ECD chromatogram of pork fat contaminated with PCBs (sum of 7 marker PCBs of 450 ng/g fat) and DDTs (concentrations of *p,p´*-DDE and *p,p´*-DDT of 3,000 and 500 ng/g fat, respectively).

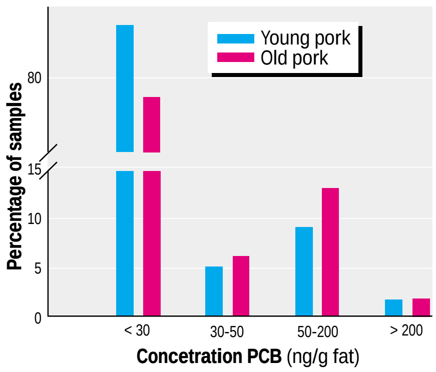

Figure 2. Concentrations of PCBs in Belgian export meat (n = 1,466).

Table 1. PCB levels in 1,850 samples of Belgian export meat.

Type of meat	< 30 ng PCBs/g fat %	< 30 ng PCBs/g fat Mean ± SD	> 30 to < 50 ng PCBs/g fat %	> 30 to < 50 ng PCBs/g fat Mean ± SD	> 50 to < 200 ng PCBs/g fat %	> 50 to < 200 ng PCBs/g fat Mean ± SD	> 200 ng PCBs/g fat %	> 200 ng PCBs/g fat Mean ± SD
Chicken (n = 384)	86.2	11.7 ± 6.1	2.9	39.7 ± 6.1	9.1	88.3 ± 33.7	1.8	244 ± 48
Pork (n = 1,466)	81.9	12.0 ± 6.3	5.7	39.9 ± 5.0	10.6	91.9 ± 39.9	1.8	356.7 ± 161

Differences between mean PCB concentrations in chicken and pork for the same category were not statistically significant ($p > 0.05$).

We also measured DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its main metabolite, DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], expressed here as DDTs, in 750 of the export meat samples. Percentage of samples with different concentrations of DDTs, together with mean and standard deviation, are shown in Table 2. In some samples, concentrations of DDTs exceeded the maximum limit set by the European Union of 1,000 ng/g fat (*6*), and were as high as 8,535 ng DDTs/g fat. Concentrations of PCBs and DDTs were not correlated (r^2 = 0.04), indicating different sources of contamination. In some cases low PCB concentrations were associated with high concentrations of DDTs. The mean DDTs/PCBs ratio was 1.9 ± 2.0.

Animal Food Chain

The data presented above indicate the existence in the animal food chain of a diffuse background contamination from PCBs and DDTs. To identify the sources of this background contamination, we measured fish flour (n = 6) and grains (n = 15) used in animal feed production for amounts of PCBs and DDTs (Figures 3 and 4). Fish flour contained a mean of 126 ± 121 ng PCBs/g fat, 25 ± 17 ng DDT/g fat, and 86 ± 71 ng DDE/g fat, and a mean DDT/DDE ratio of 0.33 ± 0.09. Fish flour from Peru showed significantly lower concentrations of PCBs and DDTs than did the fish flours originating from northern European countries (Figure 3). Grains for incorporation into animal feed (mostly imported) contained a mean of 0.84 ± 0.21 ng PCBs/g and 0.33 ± 0.55 ng DDTs/g product, with widely varying DDT/DDE ratios of 0.034 to 92.

Taking into account the amount of animal feed that pigs and chickens eat before being slaughtered, their life span, and their body fat content (*7*), we can calculate that grains contribute 15.4 ng/g to PCB concentration in the pork body fat, and fish flour contributes 1.1 ng/g. In chicken, grains contribute 14.0 ng/g to the PCB concentration in the body fat, and fish flour contributes 6.2 ng/g. Thus, the grains and fish flour in animal feed account for 16.5 ng PCBs/g fat in pork and 20.2 ng PCBs/g fat in chicken, and can explain the concentrations found in most of the meat samples (with concentrations of PCBs lower than 30 ng/g fat).

The PCB content of grains and fish flour does not explain the moderately elevated to high PCB concentrations (50 ng/g fat and higher) observed in 12.1% of the meat samples analyzed. However, in animal feed for both chicken and pork, variable amounts of fat (on average 6.5% for chicken and 3% for pork) are incorporated in addition to grains and fish flour (*8*).

In Belgium approximately 20 companies collect residual animal fat from slaughterhouses and melt it into a homogeneous substance that is sold to animal food producers. It seems likely that this recycled fat sometimes contains considerable amounts of PCBs and that its use might be responsible for the higher PCB levels observed in 12.1% of the meat samples included in this study.

The ratio between DDT and DDE diminishes over time due to metabolism and offers some clues concerning the nature of the pollution source. A value close to 0.3 (mean DDT/DDE ratio observed in analyzed fish flours) points to fish flour as the primary source of feed contamination with DDTs. A DDT/DDE ratio higher than 0.43, the highest ratio observed for fish flour, was found in some of the feed ingredients analyzed (Figure 4). Moreover, such high ratios were seen in 30.6% of meat samples with a concentration of DDTs higher than 20 ng/g fat. These findings indicate recent contamination of animal feed with DDT due to large-scale incorporation of contaminated grains imported from countries where DDT is still legally in use (e.g., against malaria) or has very local illegal use. This last hypothesis is sustained by extremely high values (7,000 and 1,500 ng/g fat for DDE and DDT, respectively) observed in meat samples from one farm.

The levels of background contamination with PCBs in Belgium are higher than those measured in Sweden (*9*). In pork, for 1991–1997, after excluding three samples (out of 490) with abnormally high concentrations, Glynn et al. (*9*) found PCB 153 concentrations with annual means between 3.1 and 1.2 ng/g fat, with a mean over 7 years of 1.9 ng/g fat. In Belgium, except for the most contaminated samples (0.6%), pork for export contained 25.2 ng PCBs/g fat. Assuming a mean of 0.3 for the ratio between PCB 153 and total PCB content (calculated from the 1,466 pork samples), the concentration of PCB 153 in Belgian export pork would be 8.5 ng/g fat. In these conditions, the Belgian pork would contain 4.5 times more PCBs than the Swedish pork. If feed ingredients had been the sole sources of PCB contamination in pork (previously calculated to account for 16.5 ng PCBs/g fat), the Belgian pork would still contain 2.9 times more PCBs than the Swedish pork. The higher concentrations in Belgian meat might be due at least partly to the common practice of incorporating

Table 2. Concentrations of DDTs in export Belgian meat (n = 750).

Concentration (ng DDTs/g fat)	Percentage of samples	Mean ± SD DDT (ng/g fat)	Mean ± SD DDE (ng/g fat)
< 20	93.5	2.4 ± 1.8	12.1 ± 3.9
20–50	4.8	7.3 ± 4.6	24.3 ± 7.0
50–1,000	1.4	61.4 ± 80.3	83.6 ± 61.2
> 1,000	0.3	1,011.5 ± 709.2	5,021 ± 2,829.8

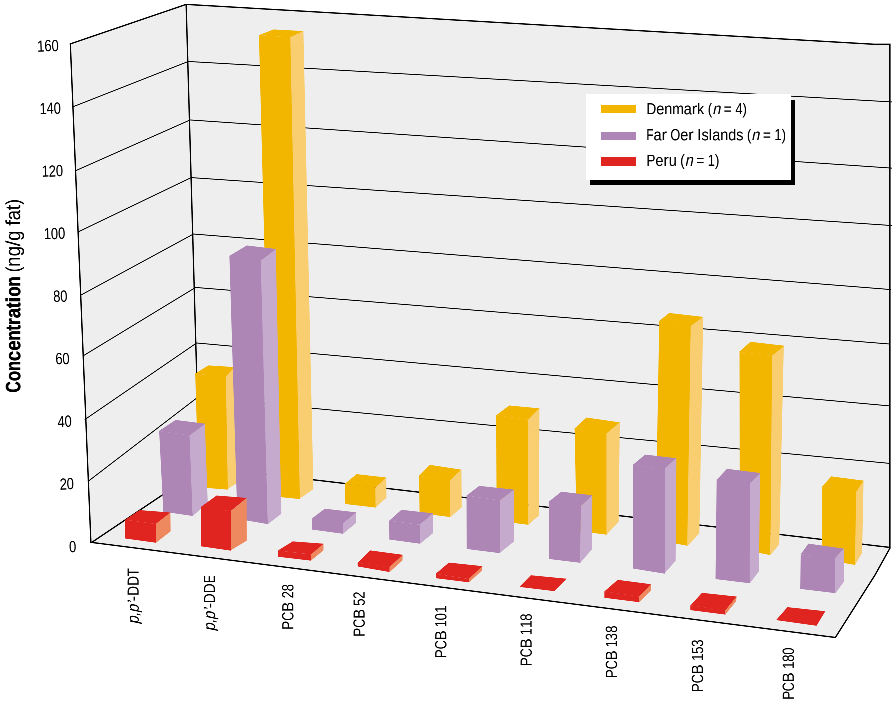

Figure 3. Organochlorine distribution in imported fish flour.

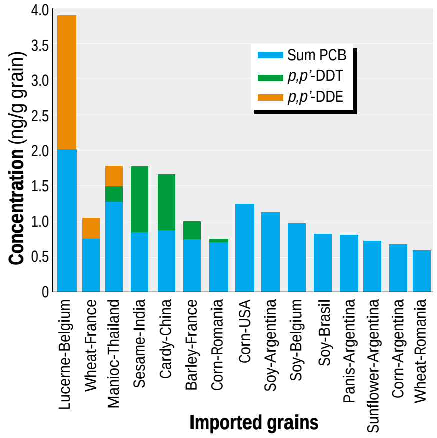

Figure 4. Organochlorine distribution in imported grains.

recycled fat of slaughtered animals (already containing a certain load of PCBs) into animal feed.

Conclusion

Although the question of the carcinogenicity (as well as other health effects) of PCBs and DDTs in humans is far from being settled (*10*), there is a high concern for the continuous decrease of their concentrations in the food chain and humans. Instead of focusing only on accidental contamination, public policy should also aim to eliminate as much as feasible the many existing sources of diffuse contamination responsible for most of the human body burdens. Together with dairy products and fish, animal meat is considered one of the most important sources of organochlorine pollutants in humans. Because concentrations of PCBs and DDTs are far below the existing tolerance limits in almost 90% of the meat samples analyzed, it appears feasible and reasonable to lower the tolerance limits for these hazardous substances without further delay.

Our results demonstrate that the use of at least some types of recycled fat should be banned. Rigorous control of organochlorines in the animal feed production is a cost-efficient way to avoid large-scale feed contamination. In addition, public health would require a permanent monitoring of organochlorines in animals used for human consumption and in humans because of their top position in the food chain.

References and Notes

1. Bernard A, Hermans C, Broeckaert F, De Poorter G, De Cock A, Houins G. Food contamination by PCBs and dioxins. Nature 401:231–232 (1999).
2. Tanabe S, Kannan N, Wakimoto T, Tatsukawa R. Isomer-specific determination and toxic evaluation of potentially hazardous coplanar PCBs, dibenzofurans and dioxins in the tissues of "Yusho" PCB poisoning victim and in the causal oil. Toxicol Environ Chem 24:215–231 (1989).
3. Beernaert H, De Poorter G. Intercomparison Study of PCBs in Feedstuffs, Animal Fat and Foodstuffs. Brussels:Belgian Ministries of Health and Agriculture, 1999.
4. Moniteur Belge. Public Law 99-1793 (Belgium), 13 June 1999.
5. Hansen LG, Welborn ME. Distribution, dilution and elimination of PCB analogs in growing swine. J Pharm Sci 66:497–501 (1977).
6. European Union Council Directive EEC 93/57. Brussels, Belgium: European Union Council, 1993.
7. Leemans M. Carry-over of PCBs Via Food in Animals [Ph.D. thesis]. Ghent, Belgium:University of Ghent, 1999.
8. Belgian Ministry of Agriculture. Personal communication.
9. Glynn AW, Wernroth L, Atuma S, Linder CE, Aune M, Nilsson I, Darnerud PO. PCB and chlorinated pesticide concentrations in swine and bovine adipose tissue in Sweden 1991–1997: spatial and temporal trends. Sci Total Environ 246:195–206 (2000).
10. Cogliano VJ. Assessing the cancer risk from environmental PCBs. Environ Health Perspect 106:317–323 (1998).